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gp100 gene. This fragment was digested with *EcoRV* and *XhoI* and cloned into *EcoRV/XhoI* digested NVQH6MC5#10 generating plasmid C5H6MELgp100 #5 which contains the gp100 gene linked to the H6 promoter.

The gp100 gene in plasmid C5H6MELgp100 #5 was sequenced using  
 5 custom primers. A 65bp deletion was found in this clone and shown to be present in pCDNA3-gp100. Plasmid PCR1I-gp100 was used in PCR with oligonucleotides MELgp05(5'-CCC-ATC-TGG-CTC-TTG-GTC-3') (SEQ.ID.NO. 115) and MELgp13 (5'-TGA-CAT-CTC-TGC-CAG-TGT-GGT-3') (SEQ.ID.NO. 116) to generate a 0.6kb fragment. This fragment was digested with *Bam*HI and  
 10 *Asp*718 and ligated to a 6.5kb *Asp*718/*Bam*HI (partial) fragment from C5H6MELgp100 #5 generating plasmid C5H6MELgp100 which contains the entire gp100 gene under the control of the H6 promoter.

Pre-existing plasmid pC5H6MELgp100 was used as template for site directed mutagenesis of the two CTL epitopes beginning at amino acids 209 and  
 15 280, respectively. Primers used were:

209-A

GCT CAG CCT TCA CCA TTA TGG ACC AGG TGC CTT TCT CC  
 (SEQ.ID.NO.117)

209-B

20 GGA GAA AGG CAC CTG GTC CAT AAT GGT GAA GGC TGA CG  
 (SEQ.ID.NO.118)

280-A

GAG CCT GGC CCA GTC ACT GTT CAG GTG GTC CTG CAG GC  
 (SEQ.ID.NO.119)

25 280-B

GCC TGC AGG ACC ACC TGA ACA GTG ACT GGG CCA GGC TC  
 (SEQ.ID.NO.120)

A section containing the modified epitopes was sequenced and isolated as a 440 bp *NcoI/Mlu*N1 fragment. This fragment was ligated into

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pC5H6MELgp100 digested with *Nco*I and *Mlu*NI, creating a plasmid with the complete gp100 with the modified epitopes 209-2M and 280-9V.

Sequence data revealed a G to C substitution at bp# 10, changing a.a. # 4 from a Valine to a Leucine. This was corrected by PCR using the following primer pair;

MEL25

GCT CCG GGA TCC CCG GCG ATG GTA GAC AGT CAC TTC CAT CGT GTG  
TGT GCC CAG CAT TG (SEQ.ID.NO.121)

MEL27

10 ATC GCG ATA TCC GTT AAG TTT GTA TCG TAA TGG ATC TGG TGC TAA  
AAA GAT GCC TTC TT (SEQ.ID.NO.122)

MEL25 changes bp# 549 from a C to a G destroying the unique *Nco*I site for easier screening. It does not change the amino acid.

The resulting PCR fragment was digested with *Bam*HI and *Eco*R5 and replaced the equivalent fragment correcting the error. The resulting plasmid is pC5gp100-M which is shown in Figure 3 (SEQ.ID.NO.123).

#### Genetic modification of the recipient:

Recombination between donor plasmid pC5gp100M and ALVAC(2) rescuing virus generated recombinant virus vCPI584, which contains the vaccinia H6 promoted modified human gp100 in the C5 locus.

### **EXAMPLE 3**

#### Screening for the identification and purification of recombinant organisms:

The aspects of screening for the identification and purification of a recombinant organism of the present invention is set out below.

25 (1) Plaque purification was done using *in situ* plaque hybridization (Piccini *et al.*, Methods of Enzymol. 153:545 (1987)) was used to identify recombinant viruses and to demonstrate purity of final virus preparations. *In situ* plaque hybridization analysis was performed with radiolabelled probes specific for the gp100 construct (a 580 bp fragment) and the C5 insertion locus.

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(2) Restriction analysis: Viral genomic DNA was isolated from cells infected with ALVAC parent or ALVAC(2)-gp100M (vCP1584). The genomic DNA was digested with restriction endonucleases (*Hind*III, *Pst* I or *Bam*HI). The resultant DNA fragments were fractionated by electrophoresis through an agarose gel and visualized by ethidium bromide staining. The insertion of the mod gp100 expression cassette at the C5 locus was confirmed.

(3) Immunoprecipitation analyses: These were performed using radiolabeled lysates derived from uninfected HeLa cells or cells infected with either ALVAC parental virus, ALVAC-gp100 (vCP1465) or ALVAC(2)-gp100M (vCP1584) as described previously (Taylor *et al.* J. Virol. 64:1441 (1990)). Briefly, HeLa cell cultures were infected at an m.o.i. of 10 pfu/cell in methionine-free media supplemented with [<sup>35</sup>S]-methionine (35uCi/ml). At 18 hrs. post infection, cells were lysed. Immunoprecipitation was performed using a rabbit anti-gp100 serum (AZN-LAM, received from M. Schreurs University of Nijmegen, Netherlands). Immunoprecipitates were fractionated on a 10% SDS-Polyacrylamide gel. The gel was fixed and treated for fluorography with 1M Na-salicylate for 1/2 hr. The dried gel was exposed to Kodak XAR-2 film to visualize the protein species. Results with anti-gp100 demonstrate expression of gp100 in ALVAC-gp100 infected HeLa cells but not for parentally infected cells. (See Figure 6)

(4) Western Blot. HeLa cells were infected for 18 hours at a multiplicity of 10 pfu/cell with ALVAC(2)-gp100M (vCP1584), ALVAC-gp100 (vCP1465) or ALVAC. Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose. The blot was incubated with AZN-LAM (1/5000 dilution) followed by HRP conjugated swine anti-rabbit utilizing the enhanced chemiluminescence (ECL) detection method (Amersham). Results demonstrate expression of full length gp100 in ALVAC-gp100 and ALVAC(2)-gp100M infected cells. (See Figure 7).

(5) Plaque immunoscreen analysis. This was performed on vCP1584 material to determine phenotypic stability of the virus upon passaging. The

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phenotypic stability of production batch material of ALVAC-gp100M (vCP1584) was analyzed by an immunological plaque assay which measures expression of the inserted genes at the plaque level. The assay utilizing permeabilized cells for detection of intracellular as well as surface expression of Hgp100mod was chosen  
5 for this test.

Test and control reagents (ALVAC(2)-gp100M (vCP1584) and ALVAC standard and ALVAC-gp100M, respectively) were plated on CEF monolayers under agarose at dilutions resulting in 40-200 plaques per 60 mm dish. 120 hours after incubation at 37°C, the infected monolayers were processed by plaque  
10 immunoassay for detection of internal expression of gp100M. Positive and negative plaques were counted for test and control samples. The primary antibody used was Monoclonal Anti-HMB50 at 1:800 dilution. A secondary antibody used was horse radish peroxidase (HRP)-conjugated rabbit anti-mouse antiserum diluted 1:500.

15 The result of analysis of internal expression of Human modified gp100 by individual plaques produced by (vCP1584) is presented in Table 1.

The result demonstrates that 98.7% of the plaque population of ALVAC-gp100M is expressing gp100M indicating that ALVAC-gp100M is phenotypically stable.

20 Results of the plaque immunoscreen analysis demonstrate that ALVAC(2)-gp100M is phenotypically stable with respect to expression of gp100.

(6) Nucleotide sequence analysis. This was performed on vCP1584 to validate the nucleotide sequence of the H6-promoted melanoma gp100M cassette. The sequence analysis revealed no nucleotide differences relative to the  
25 expected sequence, thus no mutations were introduced during the production of vCP1584. In order to carry out this analysis, a pool of plasmid clones containing a 2.2 kb PCR-derived fragment (encompassing the H6-promoted melanoma gp100M cassette), generated from vCP1584 genomic DNA was used.

pBS/1584 was generated by pooling 9 positive clones obtained by the  
30 ligation of a 2.2 kb PCR fragment (containing the H6-promoted melanoma

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gp100M cassette from vCP1584), into pBS-sk-(Stratogene). The 2.2 kb PCR fragment was derived from vCP1584 genomic DNA with the oligonucleotide primers, IDC5-1 and IDC5-2 (Figure 5). The nucleotide sequence of the oligonucleotide primers used to sequence pBS/1584 are listed in Figure 5.

5 **EXAMPLE 4**

This example provides results from injection in cynomolgus monkeys of modified gp100 molecules.

**Methods and Experimental Design**

**Test System**

- 10 Cynomolgus monkeys (Macaca fascicularis) purpose bred animals.  
Supplier: Siconbrec "Simian Conservation Breeding & Research Center Inc.",  
Fema Building, 44 Gil Puyat Avenue Makati, Metro Manila, Philippines.  
Number of animals in the study: 12 (6 males and 6 females).  
Age at initiation of treatment: 26 to 38 months.
- 15 - Body weight range at initiation of treatment (day -1):  
- males: 1.73 to 2.34 kg  
- females: 1.71 to 2.65 kg.
- Animal Husbandry**
- Housing: one air-conditioned room;
- 20 - temperature: 19 to 25°C (target range),  
- relative humidity: >40%  
- air changes: minimum 8 air changes per hour,  
- lighting cycle: 12 hours light (artificial)/12 hours dark.  
- Caging: animals were housed singly in stainless steel mesh cages
- 25 (approximately 540 x 810 x 760 mm).  
- Diet: expanded complete commercial primate diet (Mazuri diet, Special Diet Services Ltd., Witham, Essex, CM8, 3AD, Great Britain) analyzed for chemical and bacterial contaminants.  
Quantity distributed: 100g diet/animal/day.
- 30 In addition, animals received fruit daily (apple or banana)

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Animals were fasted for at least 16 hours before blood sampling for clinical laboratory investigations and before necropsy.

- Water: drinking water *ad libitum* (via bottles).

- Contaminants: no known contaminants were present in diet or water at levels which might have interfered with achieving the objective of the study.

#### **Pre-Treatment Procedures**

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.

- Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

#### **Experimental Design**

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.

- Animals were assigned to the treatment groups shown in Table 2. The dose levels administered were shown in Table 3.

#### **Administration of the Test/Control Articles**

##### Group 1 and 2 Animals

- Method of administration: injection in the left inguinal lymph node. Animals were lightly anaesthetized before each administration by an intramuscular injection of ketmine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétôquinol, Lure, France).

##### Group 3

- Route: subcutaneous.

- Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétôquinol, Lure, France). Animals were

- also lightly anaesthetized before each administration by an intramuscular

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injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 4.

5 **ELISPOT Analysis**

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFN $\gamma$  assay was used in order to measure IFN $\gamma$  production from T lymphocytes obtained from the monkeys in response to gp100 antigens.

10 **Materials and Methods**

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFN $\gamma$  antibodies/G-ZA 1 mg/mL.

- 15 Detection antibodies: MABTECH monoclonal anti-IFN $\gamma$  antibodies/7-B6-1-biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjugate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate kit/ref: 170-64 32.

20 **Coating**

Place 100  $\mu$ L per well of capture antibodies at 1  $\mu$ g/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

**Saturation**

- 25 Place 200  $\mu$ L per well of RPMI supplemented with 10% FCS, non essential amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

**Test**

- Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific stimulus (PMA-Iono). The pooled peptides used in this Example to stimulate IFN- $\gamma$
- 30

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production were derived from gp100 and are illustrated in Tables 5 to 8. The final volume of each sample is 200  $\mu$ L. Incubate 20 hours at 37°C.

Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Detection

- 5 Place 100  $\mu$ L per well of detection antibodies at 1  $\mu$ g/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Reaction

- Place 100  $\mu$ L per well of Extravidin-PA conjugate diluted 1/6000 in 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

#### Substrate Addition

- Place 100  $\mu$ L per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each spot corresponds to an individual IFN- $\gamma$  secreting T cell.

#### Results

- 20 The results of the ELISPOT analysis are shown in Figures 8-11. The results demonstrate that of the animals tested, 2 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

#### 25 ELISA Analysis

- The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5 $\mu$ g/well. Plates were placed at 4°C overnight. Plates were then washed and blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2)

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was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were  
5 diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was  
10 added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H<sub>2</sub>O<sub>2</sub> in substrate buffer (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450nm.

## 15 Results

The results of the above experiment are presented in Table 9 and in Figure 12. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous injection of the  
20 ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 12, both types of injection of the antigens induced a significant humoral response to the antigen.

In summary, the results of this Example demonstrate that injection of a  
25 tumor antigen according to the invention induces both a significant humoral and cell mediated response.

## EXAMPLE 5

This example presents data obtained from human melanoma patients primed with ALVAC(2)-gp100M and boosted with modified gp100 peptides  
30 (g209-2M and g280-9V).

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## Immunization Protocol

5 Patients were immunized subcutaneously in a prime-boost schedule with ALVAC(2)-gp100M ("prime"; lyophilized ALVAC(2)-gp100M resuspended in 1 ml of 0.4% NaCl; 0.5 ml injections (approximately  $0.5 \times 10^{7.00}$  CCID<sub>50</sub> per injection)) and peptides g209-2M and g280-9V ("boost"; 1000µg/peptide in 1 ml total volume per week (0.2 ml/injection per day x 5 days)). All patients: 1) were HLA-A0201 positive; 2) were between 18 and 70 years of age; 3) exhibited pathologically confirmed malignant melanoma; 4) demonstrated immunocompetence by reactivity to at least 2 or more out of 7 Cell Mediated Immunity (CMI) skin tests; 5) had blood hematology and chemistry values within the following ranges:

## I) Hematology:

Hemoglobin	> 100g/L
Granulocytes	> $2.0 \times 10^9$ /L
Lymphocytes	> $1.5 \times 10^9$ /L
Platelets	> $100 \times 10^9$ /L

## II) Chemistry:

Serum creatinine	< 150 µmol/L
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Serum total bilirubin	< 30 µmol/L
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AST, ALT, and ALP	Must be < 2x the normal upper limit or < 5x the normal upper limit if due to liver metastases.
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Patients "primed" with ALVAC(2)-gp100M on weeks 1, 4 and 7; "boosted" with peptides on weeks 10 and 13.

**ELISPOT Analysis:** These results are present in Tables 10 and 11. Peripheral Blood Mononuclear Cells ("PBMNC") were isolated by density centrifugation

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over Ficoll gradients. Cells were bulk-cultured at  $3 \times 10^6$ /ml in AIM-V media along with a mixture of g209-2M and g280-9V or the HLA-A\*0201 binding Flu peptide (all at 50  $\mu$ g/ml) for 8 days. IL-2 was added on days 3 and 5 of culture. On day 9, cells were harvested, counted and  $2 \times 10^5$  cells/well plus 50 U/ml IL-2, with and without the respective peptides, were plated in nitrocellulose membrane containing ELISPOT plates that had been precoated with anti-INF- $\gamma$  antibodies. The plates were developed after 48 hours of culture. The numbers reported are the differences between the average of two wells restimulated with peptide and IL-2 and two wells treated only with IL-2.

Responses are the number of spots (counted by the electronic ELISPOT reader but confirmed in most cases by manual counting) per  $2 \times 10^5$  PBMNC. The number of CD8+ T cells was not routinely determined but is typically 2-5-fold less than this number.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein, are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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**TABLE 1**

Analysis of expression of gp100 antigen by ALVAC-gp100M

	Human gp100M			
	Positive Plaques	negative plaques	total # of plaques	% positive
ALVAC std.	0	571	571	0
vCP1584	387	0	387	100
ALVAC gp100mod L	875	11	886	98.7

**TABLE 2**

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod: days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

\*209(2M)-IMDQVPFSY (SEQ.ID.NO.124); 290(9V) YLEPGPVTV (SEQ.ID.NO.125)

- 5
- Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
  - Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

**TABLE 3**

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: $0.25 \times 10^{7.4}$ CCID 50 ALVAC (2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID50	0.250
	Dose: 200 $\mu$ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVT (290(9V)) (100 $\mu$ g each)	0.2
3	Saline (NaCl 0.9%)	0.250
	ALVAC(2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID 50	0.250
	Dose: 200 $\mu$ g (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVT (290(9V)) (100 $\mu$ g each)	0.2

**TABLE 4**

Days	Sites used
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

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**TABLE 5**

Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGATK	SEQ.ID.NO.3
1330	GALLAVGATKVPRNQ	SEQ.ID.NO.4
1331	VGATKVPRNQDWLG	SEQ.ID.NO.5
1332	VPRNQDWLGVSQRLR	SEQ.ID.NO.6
1333	DWLGVSQRLRTKAWN	SEQ.ID.NO.7
1334	SQRLRTKAWNRLYP	SEQ.ID.NO.8
1335	TKAWNRLYPEWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQRLDC	SEQ.ID.NO.10
1337	EWTEAQRLDCWRGGQ	SEQ.ID.NO.11
1338	QRLDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNDGP	SEQ.ID.NO.13
1340	VSLKVSNDGPTLIGA	SEQ.ID.NO.14
1344	IALNFFGSKVLPDG	SEQ.ID.NO.15
1345	PGSQKVLDPGQVIWV	SEQ.ID.NO.16
1346	VLPDGQVIWVNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNTIINGSQVWGGQP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGGQPVYPQETDDAC	SEQ.ID.NO.21
1351	VYPQETDDACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGSWSQ	SEQ.ID.NO.24
1355	GSWSQKRSFVYVWKT	SEQ.ID.NO.25
1356	KRSFVYVWKTWGQYW	SEQ.ID.NO.26
1357	YVWKTWGQYWQVLGG	SEQ.ID.NO.27
1358	WGQYWQVLGGPV5GL	SEQ.ID.NO.28
1359	QVLGGPV5GLSIGTG	SEQ.ID.NO.29

**TABLE 6**

Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHTME	SEQ.ID.NO.31
1362	RAMLGTHTMEVTVYH	SEQ.ID.NO.32
1363	THTMEVTVYHRRGSR	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSSS	SEQ.ID.NO.35
1366	SYVPLAHSSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPFVSVS	SEQ.ID.NO.37
1369	DQVPFVSVSQRLAL	SEQ.ID.NO.38
1370	SVSVSQRLALDGGNK	SEQ.ID.NO.39
1372	DGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPGY	SEQ.ID.NO.42
1375	ALQLHDPGYLAEAD	SEQ.ID.NO.43
1379	DPGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVHTY	SEQ.ID.NO.45
1381	SRALVVHTYLEPGP	SEQ.ID.NO.46
1382	VHTYLEPGPVTQV	SEQ.ID.NO.47
1383	LEPGPVTQVVLQAA	SEQ.ID.NO.48
1384	VTAQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVP GTT	SEQ.ID.NO.51
1388	VP GTTDGHRPTAEAP	SEQ.ID.NO.52
1389	DGHRPTAEAPNTTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTEVVGTT PGQA	SEQ.ID.NO.55
1393	EVVGTTPGQAPTAEP	SEQ.ID.NO.56

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**TABLE 7**

Peptide Pool #3

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSTGTT	SEQ.ID.NO.57
1395	PTAEPSTGTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VQVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAPVQMPTAE	SEQ.ID.NO.61
1399	TAPVQMPTAESTGMT	SEQ.ID.NO.62
1400	MPTAESTGMTPEKVP	SEQ.ID.NO.63
1401	STGMTPEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGMTPEAEV	SEQ.ID.NO.68
1408	SIVVLSGTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TTARELPIPEPEGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPLLDG	SEQ.ID.NO.77
1417	TGSLGPLLDGTATLR	SEQ.ID.NO.78
1418	PLLDGTATLRLVKRQ	SEQ.ID.NO.79
1419	TATLRLVKRQVPLDC	SEQ.ID.NO.80
1420	LVKRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGSFSV	SEQ.ID.NO.82
1422	VLYRYGSFSVTLDIV	SEQ.ID.NO.83

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**Table 8****Peptide Pool #4**

Peptide	Sequence	SEQ.ID.NO.
1424	TLDIVQGIESAEILQ	SEQ.ID.NO.84
1425	QGIESAEILQAVPSG	SEQ.ID.NO.85
1426	AEILQAVPSGEGDAF	SEQ.ID.NO.86
1427	AVPSGEGDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGL	SEQ.ID.NO.88
1429	ELTVSCQGGLPKEAC	SEQ.ID.NO.89
1430	CQGGLPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQL	SEQ.ID.NO.92
1434	PAQRLCQPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGSGTY	SEQ.ID.NO.96
1441	LADTNSLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPGQE	SEQ.ID.NO.98
1443	STQLIMPGQEAGLQ	SEQ.ID.NO.99
1444	MPGQEAGLQVPLIV	SEQ.ID.NO.100
1445	AGLQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVLASLIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPQL	SEQ.ID.NO.103
1451	MKQDFSVPQLPHSSS	SEQ.ID.NO.104
1452	SVPQLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSPIG	SEQ.ID.NO.107
1455	PRIFCSPIGENSPL	SEQ.ID.NO.108

TABLE 9

Monkey #	DAY (mOD/min)			
	0	57	68	96
1	3	5	2	2
2	4	6	12	10
3	7	6	10	8
4	7	6	8	8
5	5	9	20	15
6	11	8	10	12
7	11	23	51	30
8	7	30	70	22
9	1	7	5	3
10	2	6	6	4
11	3	7	14	8
12	6	9	15	6

**TABLE 10****Gp100-specific responses to g209-2M and g280-9V\***

Patient	Pre 1 <sup>st</sup> Injection	Pre 2 <sup>nd</sup> Injection	Pre 3 <sup>rd</sup> Injection	Pre 4 <sup>th</sup> Injection	Pre 5 <sup>th</sup> Injection	4 wks post vaccination
#1	0	0	0	ND	ND	2±1.4
#2	0	14±2.8	54±6.4	16±7.8	ND	ND
#3	0	0	ND	ND	ND	ND
#4	0	0	24±13.4	1±2.1	ND	ND
#5	ND	6±6.4	ND	ND	ND	ND

5

**TABLE 11**10 **Flu-peptide specific responses\***

Patient	Pre 1 <sup>st</sup> Injection	Pre 2 <sup>nd</sup> Injection	Pre 3 <sup>rd</sup> Injection	Pre 4 <sup>th</sup> Injection	Pre 5 <sup>th</sup> Injection	4 wks post vaccination
#1	>150	ND	>70	ND	ND	12.5
#2	ND	0	24	0	ND	ND
#3	23.5	7	ND	ND	ND	ND
#4	0	29	13.5	11.5	ND	ND
#5	ND	>200	ND	ND	ND	ND

\* ND signifies that the values were not determined for the sample.

## WE CLAIM:

1. An isolated and purified modified gp100 molecule capable of modulating an immune response in an animal.
- 5 2. A molecule according to claim 1 having a nucleic acid sequence shown in Figure 1 (SEQ.ID.NO.1).
3. A molecule according to claim 1 or 2 which comprises:
  - 10 (a) a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1) wherein T can also be U;
  - (b) nucleic acid sequences complementary to (a);
  - (c) nucleic acid sequences which are homologous to (a) or (b);
  - (d) a fragment of (a) to (c);
  - 15 (e) a nucleic acid which will hybridize to (a) to (d) under stringent hybridization conditions; and
  - (f) a nucleic acid molecule differing from any of the nucleic acids of (a) to (d) in codon sequences due to the degeneracy of the genetic code.
- 20 4. The nucleic acid of any one of claims 1-3 wherein the nucleic acid is selected from the group consisting of viral nucleic acid, plasmid, bacterial DNA, naked/free DNA, and RNA.
5. A viral nucleic acid of claim 4 wherein the virus is selected from  
25 adenovirus, alphavirus or poxvirus.
6. A poxvirus of claim 5 which is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
- 30 7. The poxvirus of claim 6 which is ALVAC.

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8. A composition comprising the nucleic acid of any one of claims 1-7 and a pharmaceutically acceptable diluent or carrier.
- 5 9. A composition according to claim 8 further comprising an adjuvant.
10. A cell comprising a nucleic acid according to any one of claims 1-7 wherein the cell expresses a polypeptide encoded by the nucleic acid.
- 10 11. A cell according to claim 10 wherein the cell is an antigen-presenting cell.
12. A cell according to claim 10 wherein the cell is a dendritic cell.
13. A recombinant virus comprising a virus into which is inserted a nucleic acid according to any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, the recombinant virus causing the expression of the polypeptide in an infected cell.
- 15 14. A recombinant virus into which is inserted a nucleic acid according to any one of claims 1-7 wherein the nucleic acid encodes for a polypeptide, wherein cells infected with the said recombinant virus are capable of eliciting an immune response directly against a member selected from the group consisting of:
- 25 (1) the polypeptide;
- (2) a fragment of the polypeptide;
- (3) a cell expressing the polypeptide or a fragment thereof; or
- (4) cells binding the protein or fragment thereof.
15. A recombinant virus according to claim 13 or 14 selected from
- 30 adenovirus, alphavirus, or poxvirus.

16. A recombinant virus according to claim 15 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
- 5 17. A recombinant virus according to claim 16 wherein the virus is ALVAC.
18. A composition comprising a recombinant virus of any one of claims 13 to 17 and a pharmaceutically acceptable diluent or carrier.
- 10 19. An isolated protein encoded by a nucleic acid molecule according to any one of claims 1-7.
20. An isolated protein having the activity of a modified gp100 protein.
- 15 21. A protein having the amino acid sequence shown in Figure 2 (SEQ.ID.NO.2).
22. A method of modulating an animal's immune system comprising administering an effective amount of a gp100 or *gp100* which has been modified.
- 20 23. A method according to claim 22 where the gp100 is gp100M.
24. A method according to claim 22 wherein the *gp100* is *gp100M*.
- 25 25. A method according to claim 24 wherein the gp100M has a nucleic acid sequence as shown in Figure 1 (SEQ.ID.NO.1).
26. A method according to claim 23 wherein the gp100M has an amino acid shown in Figure 2 (SEQ.ID.NO.2).

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27. A method of modulating an animal's immune system comprising administering to an animal in need thereof, an effective amount of a vector, into which has been inserted a *gp100* which has been modified, thereby modulating the animal's immune system.
- 5 28. A method according to claim 27 wherein the vector is administered with a lymphokine, cytokine, or a co-stimulatory molecule.
29. A method according to claim 28 wherein the cytokine is GM-CSF, IL-2,  
10 IL-12, TNF, or IFN $\gamma$ 1.
30. A method according to claim 28 wherein the molecule is a lymphokine.
31. A method according to claim 28 wherein the molecule is co-stimulatory  
15 molecule.
32. A method according to claim 31 wherein the co-stimulatory molecule is a molecule of the B7 family.
- 20 33. A method according to any one of claims 27-32 wherein the vector is an adenovirus, alphavirus or poxvirus.
34. A method according to claim 33 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
- 25 35. A method according to claim 34 wherein the poxvirus is ALVAC
36. A method for prophylactic treatment of cancer comprising administering to an animal an effective amount of a modified *gp100* or

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immunogenic fragment thereof, or a nucleic acid sequence encoding a modified *gp100* or immunogenic fragment thereof.

37. A method according to claim 36 wherein the modified *gp100* has an amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

38. A method according to claim 36 wherein the nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

39. A method according to any one of claims 36, 37 or 38 wherein the cancer is a melanoma.

40. A melanoma vaccine comprising a nucleic acid sequence encoding a modified *gp100*.

41. A vaccine according to claim 40 wherein the modified *gp100* is *gp100M*.

42. A vaccine according to claim 41 wherein the *gp100M* has the amino acid sequence as shown in Figure 2 (SEQ.ID.NO.2).

43. A modified *gp100* protein sequence which is modified to enhance its binding to MHC molecules.

44. A modified protein sequence according to claim 43 wherein the protein is *gp100M*.

45. The protein of claim 44 wherein the amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).

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46. A vaccine comprising a modified *gp100* nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting the production of antibodies in a animal to corresponding antigens.

5 47. A vaccine according to claim 46 wherein the protein corresponding to the nucleic acid sequence is *gp100M*.

48. A vaccine according to claim 46 wherein the modified *gp100* nucleic acid sequence is *gp100M*.

10

49. A vaccine according to claim 48 wherein the *gp100M* nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

50. A vaccine according to claim 47 wherein the *gp100M* has an amino acid  
15 sequence as shown in Figure 2 (SEQ.ID.NO.2).

51. A vaccine comprising a modified *gp100* nucleic acid sequence or its corresponding protein or protein fragment capable of eliciting a cellular immune response.

20

52. A vaccine according to claim 51 wherein the protein corresponding to the nucleic acid sequence is *gp100M*.

53. A vaccine according to claim 51 wherein the modified *gp100* nucleic acid  
25 sequence is *gp100M*.

54. A vaccine according to claim 53 wherein the *gp100M* nucleic acid sequence is as shown in Figure 1 (SEQ.ID.NO.1).

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55. A vaccine according to claim 52 wherein the gp100M has an amino acid sequence is as shown in Figure 2 (SEQ.ID.NO.2).
56. An immunogenic composition containing a vaccine vector encoding for  
5 a modified gp100 molecule.
57. A composition according to claim 56 wherein the modified gp100 molecule is gp100M.
- 10 58. A composition according to claim 57 wherein the modified gp100M has an amino acid sequence according to Figure 2 (SEQ.ID.NO.2).
59. A composition according to any one of claims 56, 57 or 58 wherein the vector is an adenovirus, alphavirus or poxvirus.
- 15 60. A composition according to claim 59 wherein the poxvirus is vaccinia, fowlpox, avipox, TROVAC, ALVAC, NYVAC or MVA.
61. A composition according to claim 60 wherein the poxvirus is ALVAC.
- 20 62. Immunogenic fragments of an isolated gp100M protein encoded by a nucleic acid molecule having a sequence according to SEQ ID NO. 1.

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## FIGURE 1

AGGTGCTTTG	CTGGCTGTGG	GGGCTACAAA	AGTACCCAGA	AACCAGGACT	GGCTTGGTGT
CTCAAGGCCAA	CTCAGAACCA	AAGCCTGGAA	CAGGCAGCTG	TATCCAGAGT	GGACAGAAAGC
CCAGAGACTT	GACTGCTGGA	GAGGTGGTCA	AGTGTCCCTC	AAGGTCAGTA	ATGATGGGGCC
TACACTGATT	GGTGCAAAATG	CCTCCTCTCT	TATTGCTTG	AACCTCCCTG	GAAGCCAAAA
GGTATTGCCA	GATGGGCAGG	TTATCTGGGT	CAACAATACC	ATCATCAATG	GSAGCCAGGT
GTGGGGAGGA	CAGCCAGTGT	ATCCCAGGA	AACGTACGAT	GCCTGCATCT	TCCTGATGG
TGGACCTTGC	CCATCTGGCT	CTTGGTCTCA	GAAGAGAAGC	TTTGTTTATG	TCTGGGAAGAC
CTGGGGCCAA	TACTGGCAAG	TTCTAGGGGG	CCCAGTGTCT	GGGCTGAGCA	TTGGGACAGG
CAGGGCAATG	CTGGGCACAC	ACACGATGGA	AGTGACTGTC	TACCATCGCC	GGGGATCCCG
GAGCTATGTG	CCCTCTGTCT	ATTCCAGCTC	AGCCTTCACC	ATTATGGACC	AGGTGCCTTT
CTCCGTGAGC	GTGTCOCAGT	TGCGGGCCTT	GGATGGAGGG	AACAAGCACT	TCCTGAGAAA
TCAGCCTCTG	ACCTTTGCCC	TCCAGCTCCA	TGACCCAGT	GGCTATCTGG	CTGAAGCTGA
CCCTCTCTAC	ACCTGGGACT	TTGGAGACAG	TAGTGGAAAC	CTGATCTCTC	GGGCACTTGT
GGTCACTCAT	ACTTACCTGG	AGCCTGGCCC	AGTCACTGTT	CAGGTGGTCC	TGCAGGCTGC
CATTCTCTCT	ACCTCCTGTG	GCTCCTCCCC	AGTCCAGGC	ACCACAGATG	GGCAGAGGCC
AAGTCGAGAG	GGCCCTAACA	CCACAGCTGG	CCAAGTGCT	ACTACAGAAG	TTGTGGGTAC
TACACCTGGT	CAGGCCCCAA	CTGCAGAGCC	CTCTGGAAAC	ACATCTGTGC	AGGTGCCAAC
CAGTGAAGTC	ATAAGCACTG	CACCTGTGCA	GATGCCAACT	GCAGAGAGCA	CAGGTATGAC
ACCTGAGAAG	GTGCCAGTTT	CAGAGGTCAT	GGGTACCCCA	CTGGCAGAGA	TGTCAACTCC
AGAGGGTACA	GGTATGACAC	CTGCAGAGGT	ATCAATTGTG	GTGCTTCTCT	GAACACACAGC
TGCAACAGGT	ACAACTACAG	AGTGGGTGGA	GACCACAGCT	AGAGAGCTAC	CTATCCCTGA
GCCTGAAGGT	CCAGATGCCA	GCTCAATCAT	GTCTACGGAA	AGTATTACAG	GTTCCTCTGG
CCCCCTGCTG	GATGGTACAG	CCACCTTAAG	GCTGGTGAAG	AGACAAGTCC	CCCTGGATTG
GTCTCTGTAT	CGATATGGTT	CCCTTTCCGT	CACCTTGGAC	ATTGTCCAGG	GTATTGAAG
TGCCGAGATC	CTGCAGGCTG	TGCCGTCCGG	TGAGGGGGAT	GCATTTGAGC	TGACTGTGTC
GTGCCAAGGC	GGGTTGCCCA	AGGAAGCCTG	CATGGAGATC	TCATGCCAGC	GGTGCCAGCC
CCCTGCCAG	CGGCTGTGCC	AGCCTGTGCT	ACCCAGCCCA	GCCTGCCAGC	TGGTCTGCA
CCAGATACTG	AAGGGTGGCT	CGGGGACATA	CTGCCTCAAT	GTGTCTCTGG	CTGTATACCAA
CAGCCTGGCA	GTGGTCAGCA	CCCAGCTTAT	CATGCCCTGGT	CAAGAAGCAG	GCCTTGGGCA
GGTTTCOGCTG	ATCGTGGGCA	TCTTGTCTGT	GTTGTGGCT	GTGGTCCTTG	CATCTCTGAT
ATATAGGCCG	AGACTTATGA	AGCAAGACTT	CTCCGTACCC	CAGTTGCCAC	ATAGCAGCAG
TCACTGGCTG	CGTCTACCCC	GCATCTTCTG	CTCTTGTCCC	ATTGGTGAGA	ACAGCCCCCT
CCTCAGTGGG	CAGCAGGTCT	GA			

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## FIGURE 2

```

Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
  1           5           10           15
Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
          20           25           30
Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
  35           40           45
Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
  50           55           60
Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
  65           70           75           80
Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
          85           90           95
Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
  100           105           110
Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
  115           120           125
Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
  130           135           140
Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
  145           150           155           160
Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
          165           170           175
Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
  180           185           190
Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
  195           200           205
Ile Met Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
  210           215           220
Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
  225           230           235           240
Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
          245           250           255
Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
  260           265           270
Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Val
  275           280           285
Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
  290           295           300
Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
  305           310           315           320
Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
          325           330           335
Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
  340           345           350
Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
  355           360           365

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## FIGURE 2 (CONT'D)

[illegible]

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## FIGURE 3

### Nucleotide Sequence of CSH6p100M

1-254 left C5 flanking arm  
 255-376 H6 promoter  
 377-2362 modified gp100 gene  
 2363-2534 right C5 flanking arm

```

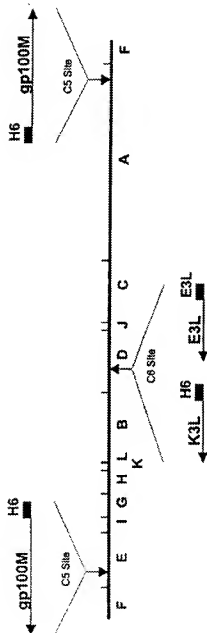
1 GGCTACTTTT CAACAAAGGA GCAGATGTAA ACTACATCTT TGAAGAAAT GGAAATCAT
61 ATACTGTTTT GGAATTGATT AAAGAAAGTT ACTCTGAGAC ACAAAGAGG TAGCTGAAGT
121 GGTACTCTCA AAGGTACGTG ACTAATTAGC TATAAAAAGG ATCGTCGACG AGCTCGAATT
181 CGGATCCGGG TTAATTAATT AGTCATCAGG CAGGGCGAGA ACGAGACTAT CTGCTCGTTA
241 ATTAATTAGA GCTTCTTTAT TCTATACTTA AAAAGTGAAA ATAAATACAA AGGTCTTTGA
301 GGGTTGTGTT AAATTGAAAG CGAGAAATAA TCATAAATTA TTTCATTATC GCGATATCCG
361 TTAAGTTTGT ATCGTAAATG ATCTGGTGCT AAAAGATATG CTTCCTTCATT TGCGTGTGAT
421 AGGTGCTTTG CTGGCTGTGG GGGCTACAAA AGTACCCAGA AACCAGGACT GGCTTGGTGT
481 CTCAGGCCAA CTCAGAACCA AAGCCTGGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
541 CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTCAGTA ATGATGGGCC
601 TACACTGATT GGTGCAAAATG CCTCCTTCTC TATTGCCITG AACTTCCCTG GAAGCCAAAA
661 GGTATTGCCA GATGGGCAGG TTATCTGGGT CAACAATACC ATCATCAATG GGAGCCAGGT
721 GTGGGGAGGA CAGGCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCATCT TCCTGATGG
781 TGGACCTTGC CCATCTGGCT CTGGGTCTCA GAAGAGAAGC TTGTGTTATG TCTGGAAGAC
841 CTGGGGCCAA TACTGGCAAG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
901 CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGACTGTC TACCATCGCC GGGGATCCCG
961 GAGCTATGTG CCTCTTGCTC ATTCAGCTC AGCCTTCACC ATTATGGACC AGGTGCCCTT
1021 CTCGCTGAGC GTGTCCAGT TGGGGGCCCT GGGTGGAGGG AACACGACTT TCCTGAGAAA
1081 TCAGCCTCTG ACCTTTGCCC TCCAGCTCCA TGACCCAGT GGCTATCTGG CTGAAGCTGA
1141 CCTCTCCTAC ACCTGGGACT TTGGAGACAG TAGTGGAAAC CTGATCTCTC GGGCACTTGT
1201 GGTCACTCAT ACTTACCTGG AGCCTGCCCC AGTCACTGTT CAGGTGGTCC TGCAGGCTGC
1261 CATTCCTCTC ACCTCCTGTG GCTCCTCCCC AGTTCAGGC ACCACAGATG GGCACAGGCC
1321 AACTGCAGAG GCCCTTAACA CCACAGCTGG CCAAGTGCTT ACTACAGAAG TTGTGGGTAC
1381 TACACCTGGT GAGGCCCAA CTGCAGAGCC CTCTGGAACC ACATCTGTGC AGTGGCCAA
1441 CACTGAAGTC ATAAGCACTG CACTGTGCA GATGCCAATC GCAGAGAGCA CAGGTATGAC
1501 ACCTGAGAAG GTGCCAGTTT CAGAGGTAT ATCAATTGTG GTGCTTTCTG GAACACAGCC
1561 AAGAGCTACA GGTATGACAC CTGCAGAGGT ATCAATTGTG GTGCTTTCTG GAACACAGCC
1621 TGCACAGGTA ACAAATACAG AGTGGGTGGA GACCACAGCT AGAGAGCTAC CTATCCAGA
1681 GCCTGAAGGT CAGATAGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
1741 CCCCCTGCTG GATGGTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
1801 TGTTCTGTAT CGATATGGTT CTTTTCCGT CACCTGGAG CATTGTAAGC TGACTGTGTG
1861 TGCCGAGATC CTGCAGGCTG TGCCGTCCGG TGAGGGGGAT GCATTGTAAGC TGACTGTGTG
1921 CTGCCAAGGC GGGCTGCCCA AGGAAGCCTG CATCGAGAT CTATCGCCAG GGTGCCAGCC
1981 CCCTGCCCAG CGCTGTGCT ACCAGGCCA GCCTGCCAGC TGGTCTGCA
2041 CCAGTACTAG AAGGGTGGCT CGGGGACATA CTGCCCTCAAT GTGCTCTGG CTGATACCA
2101 CAGCCTGSCA GTGGTCAGCA CCGAGCTTAT CATGCTGGT CATGCTGGT GTATTGAAAG
2161 GGTTCGCTG ATCGTGGGCA TCTTGCTGGT GTTGATGGCT GTGGTCTTGG CATCTCTGAT
2221 ATATAGGCGC AGACTATGTA AGCAAGACTT CTCCGTACCC CATTCGCCAG GTAGCAGCAG
2281 TCACGTGGCTG CGTCTACCCC GCATCTTCTG CTCTTGTCCT ATTGGTGAGA ACAGCCCCCT
2341 CCTCAGTGGG CAGCAGCTGT GATTTTTATC TCGAGTCTGA AATCGATCCC GGGTTTTTAT
2401 GACTAGTTAA TCAGGCCGCT TTATAAAGAT CTAAAAATGA TAATTTCTAA ATATGAAA
2461 AAAAGTACAT CATGAGCAC GCGTTAGTAT ATTTTCAAT GGAGATTAAC GCTCTATACC
2521 GTTCTATGTT TATT

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FIGURE 4

# ALVAC(2)-gp100M (vCP1584) (ALVAC XhoI Restriction Map)



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## FIGURE 5

## Oligonucleotide Primers

IDC5-1

CGT GCC ATG GCA CAC AAA AGA GGT AGC TGA A

IDC5-2

CCA GGC GGC CGC ACT AAC GCG TTG CTC ATG ATG

C5L

CAC AAA AGA GGT AGC TGA AGT

MEL 01

ATG GAT CTG GTG CTA AAA AGA

MEL 05

ACC TTG CCC ATC TGG CTC TTG

MEL 09

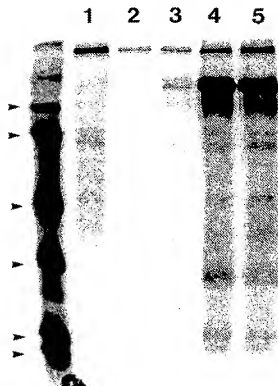
AGA TGC CAG CTC AAT CAT GTG

C5R

ATA GAT CTT TAT AAG CGG CCG

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## FIGURE 6



Molecular Weight Markers: 200, 98.6, 68, 43, 29, 18, 14 kDa

Lane 1: Uninfected HeLa cells

Lane 2: HeLa cells infected with ALVAC

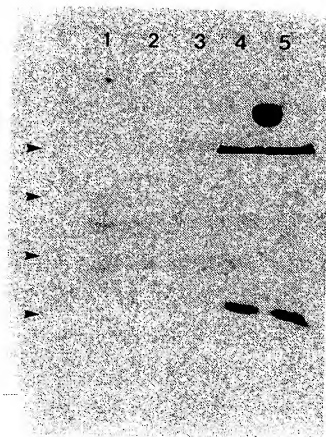
Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)

Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)

Lane 5: HeLa cells infected with ALVAC(2)-gp100M (sister of vCP1584)

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## FIGURE 7



Molecular Weight Markers: 97, 68, 43, 29 kDa

Lane 1: Uninfected HeLa cells

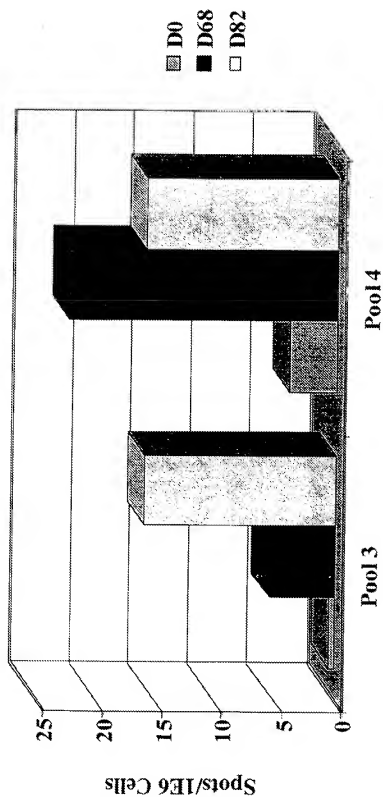
Lane 2: HeLa cells infected with ALVAC

Lane 3: HeLa cells infected with ALVAC-gp100 (vCP1465)

Lane 4: HeLa cells infected with ALVAC(2)-gp100M (vCP1584)

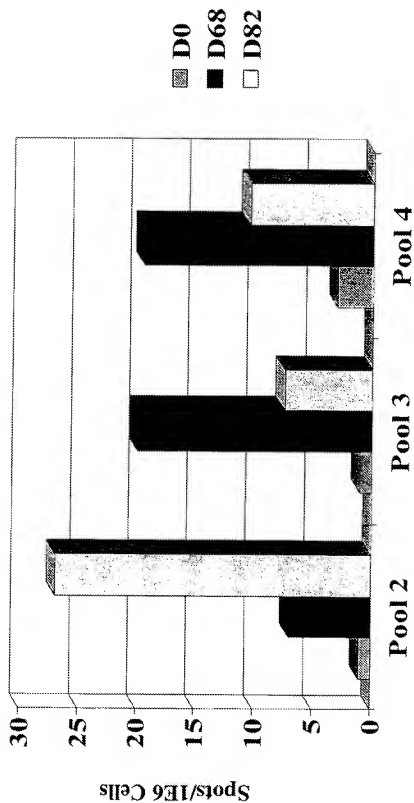
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**FIGURE 8**  
**Monkey #6 (Intranodal Administration)**



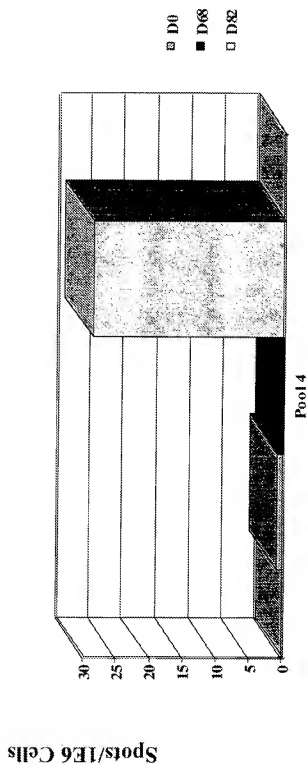
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**FIGURE 9**  
**Monkey #7 (Intranodal Administration)**



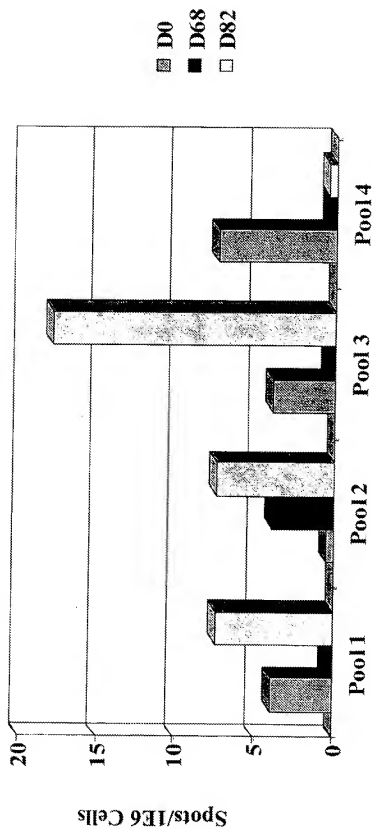
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**FIGURE 10**  
**Monkey # 11 (Subcutaneous Administration)**



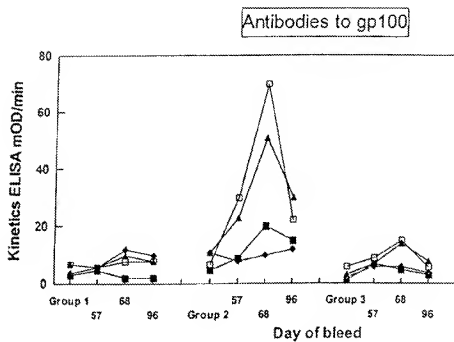
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**FIGURE 11**  
**Monkey #10 (Subcutaneous Administration)**



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FIGURE 12



## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/CA 00/01254

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 C07K14/705 C12N9/64 A61K39/00 C12N5/06 C12N7/00  
 A61K35/76 A61P35/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, CHEM ABS Data, SCISEARCH, BIOSIS, WPI Data, EPO-Internal, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>IRVINE KARI R ET AL: "Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens."            CANCER RESEARCH,            vol. 59, no. 11, 1 June 1999 (1999-06-01),            pages 2536-2540, XP002161590            ISSN: 0008-5472</p> <p>page 2536, left-hand column, line 39            -right-hand column, line 14            page 2356, right-hand column, line 47            -page 2357, left-hand column, line 1            table 1</p> <p style="text-align: center;">--- -/-</p>	<p>8,9,            13-16,            18-20,            22-24,            27-34,            36,            39-41,            43,44,            46-48,            51-53,            56,57,            59,60</p>

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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\*A\* document defining the general state of the art which is not considered to be of particular relevance

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\*O\* document relating to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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## INTERNATIONAL SEARCH REPORT

International Application No

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